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☐ 1. Document ID: US 6403056 B1

L2: Entry 1 of 18 File: USPT Jun 11, 2002

US-PAT-NO: 6403056
DOCUMENT-IDENTIFIER: US 6403056 B1

TITLE: Method for delivering bioactive agents using cochleates

DATE-ISSUED: June 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Unger; Evan C.	Tucson	AZ		

US-CL-CURRENT: 424/9.51; 424/400, 424/450, 424/502, 424/9.52

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☐ 2. Document ID: US 6315981 B1

L2: Entry 2 of 18 File: USPT Nov 13, 2001

US-PAT-NO: 6315981
DOCUMENT-IDENTIFIER: US 6315981 B1

TITLE: Gas filled microspheres as magnetic resonance imaging contrast agents

DATE-ISSUED: November 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Unger; Evan C.	Tucson	AZ		

US-CL-CURRENT: 424/9.323; 424/450, 424/9.3, 424/9.32, 424/9.321, 424/9.322, 424/9.33, 424/9.36, 424/9.37, 424/9.51, 424/9.52, 600/420

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☐ 3. Document ID: US 6200598 B1

L2: Entry 3 of 18 File: USPT Mar 13, 2001

US-PAT-NO: 6200598
DOCUMENT-IDENTIFIER: US 6200598 B1

TITLE: Temperature-sensitive liposomal formulation

DATE-ISSUED: March 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Needham; David	Durham	NC		

US-CL-CURRENT: 424/450; 424/1.21, 424/9.321, 424/9.51, 424/94.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 4. Document ID: US 6120751 A

L2: Entry 4 of 18 File: USPT Sep 19, 2000

US-PAT-NO: 6120751
DOCUMENT-IDENTIFIER: US 6120751 A

TITLE: Charged lipids and uses for the same

DATE-ISSUED: September 19, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Unger; Evan C.	Tucson	AZ		

US-CL-CURRENT: 424/9.51; 264/4, 264/4.1, 424/450, 424/502, 424/9.52, 428/402.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 5. Document ID: US 6086851 A

L2: Entry 5 of 18 File: USPT Jul 11, 2000

US-PAT-NO: 6086851
DOCUMENT-IDENTIFIER: US 6086851 A

TITLE: Pharmaceutical compositions containing interdigitation-fusion liposomes and gels

DATE-ISSUED: July 11, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Boni; Lawrence T.	Monmouth Junction	NJ		
Janoff; Andrew S.	Yardley	PA		
Minchey; Sharma R.	Monmouth Junction	NJ		
Perkins; Walter R.	Monmouth Junction	NJ		
Swenson; Christine E.	Princeton Junction	NJ		
Ahl; Patrick L.	Princeton	NJ		
Davis; Thomas S.	Valhalla	NY		

US-CL-CURRENT: 424/9.4; 264/4.1, 264/4.3, 264/4.32, 424/450, 428/402.2, 428/402.24

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 6. Document ID: US 6041252 A

L2: Entry 6 of 18 File: USPT Mar 21, 2000

US-PAT-NO: 6041252
DOCUMENT-IDENTIFIER: US 6041252 A

TITLE: Drug delivery system and method

DATE-ISSUED: March 21, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Walker; Jeffrey P.	San Diego	CA		
Bernard; Robert M.	Rancho Santa Fe	CA		

US-CL-CURRENT: 604/20; 435/173.6, 435/285.2, 604/21, 607/72

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 7. Document ID: US 5939096 A

L2: Entry 7 of 18 File: USPT Aug 17, 1999

US-PAT-NO: 5939096
DOCUMENT-IDENTIFIER: US 5939096 A

TITLE: Liposome drug-loading method and composition

DATE-ISSUED: August 17, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Clerc; Stephane	Castres			FRX
Barenholz; Yechezkel	Jerusalem			ILX

US-CL-CURRENT: 424/450

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 8. Document ID: US 5922304 A

L2: Entry 8 of 18 File: USPT Jul 13, 1999

US-PAT-NO: 5922304
DOCUMENT-IDENTIFIER: US 5922304 A

TITLE: Gaseous precursor filled microspheres as magnetic resonance imaging contrast agents

DATE-ISSUED: July 13, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Unger; Evan C.	Tucson	AZ		

US-CL-CURRENT: 424/9.3; 424/9.32, 424/9.321, 424/9.37, 424/9.52

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 9. Document ID: US 5869092 A

L2: Entry 9 of 18 File: USPT Feb 9, 1999

US-PAT-NO: 5869092
DOCUMENT-IDENTIFIER: US 5869092 A

TITLE: Prevention of leakage and phase separation during thermotropic phase transition in liposomes and biological cells

DATE-ISSUED: February 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hays; Lisa M.	Davis	CA		
Crowe; John H.	Davis	CA		
Crowe; Lois M.	Davis	CA		
Feeney; Robert E.	Davis	CA		
Oliver; Ann E.	Davis	CA		

US-CL-CURRENT: 424/450

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 10. Document ID: US 5820848 A

L2: Entry 10 of 18 File: USPT Oct 13, 1998

US-PAT-NO: 5820848
DOCUMENT-IDENTIFIER: US 5820848 A

TITLE: Methods of preparing interdigitation-fusion liposomes and gels which encapsulate a bioactive agent

DATE-ISSUED: October 13, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Boni; Lawrence T.	Monmouth Junction	NJ		
Janoff; Andrew S.	Yardley	PA		
Minchey; Sharma R.	Monmouth Junction	NJ		
Perkins; Walter R.	Monmouth Junction	NJ		
Swenson; Christine E.	Princeton Junction	NJ		
Ahl; Patrick L.	Princeton	NJ		
Davis; Thomas S.	Valhalla	NY		

US-CL-CURRENT: 424/9.4; 264/4.1, 424/1.21, 424/450, 424/9.321, 436/829, 516/102

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 11. Document ID: US 5770222 A

L2: Entry 11 of 18 File: USPT Jun 23, 1998

US-PAT-NO: 5770222
DOCUMENT-IDENTIFIER: US 5770222 A

TITLE: Therapeutic drug delivery systems

DATE-ISSUED: June 23, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Unger; Evan C.	Tucson	AZ		
Fritz; Thomas A.	Tucson	AZ		
Matsunaga; Terry	Tucson	AZ		
Ramaswami; VaradaRajan	Tucson	AZ		
Yellowhair; David	Tucson	AZ		
Wu; Guanli	Tucson	AZ		

US-CL-CURRENT: 424/450; 264/4.1, 264/4.3, 264/4.6, 424/1.21, 424/489, 424/9.321, 424/9.51, 436/829

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 12. Document ID: US 5580575 A

L2: Entry 12 of 18 File: USPT Dec 3, 1996

US-PAT-NO: 5580575
DOCUMENT-IDENTIFIER: US 5580575 A

TITLE: Therapeutic drug delivery systems

DATE-ISSUED: December 3, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Unger; Evan C.	Tucson	AZ		
Fritz; Thomas A.	Tucson	AZ		
Matsunaga; Terry	Tucson	AZ		
Ramaswami; VaradaRajan	Tucson	AZ		
Yellowhair; David	Tucson	AZ		
Wu; Guanli	Tucson	AZ		

US-CL-CURRENT: 424/450

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 13. Document ID: US 5542935 A

L2: Entry 13 of 18 File: USPT Aug 6, 1996

US-PAT-NO: 5542935
DOCUMENT-IDENTIFIER: US 5542935 A

TITLE: Therapeutic delivery systems related applications

DATE-ISSUED: August 6, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Unger; Evan C.	Tucson	AZ		
Fritz; Thomas A.	Tucson	AZ		
Matsunaga; Terry	Tucson	AZ		
Ramaswami; VaradaRajan	Tucson	AZ		
Yellowhair; David	Tucson	AZ		
Wu; Guanli	Tucson	AZ		

US-CL-CURRENT: 604/190; 424/450, 600/458

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 14. Document ID: US 5411730 A

L2: Entry 14 of 18 File: USPT May 2, 1995

US-PAT-NO: 5411730
DOCUMENT-IDENTIFIER: US 5411730 A

TITLE: Magnetic microparticles

DATE-ISSUED: May 2, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kirpotin; Dmitri	Denver	CO		
Chan; Daniel C. F.	Denver	CO		
Bunn, Jr.; Paul A.	Evergreen	CO		

US-CL-CURRENT: 424/9.322; 252/62.56, 423/633, 424/647, 428/403, 436/173, 436/806, 514/54, 514/59, 514/6, 607/100

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 15. Document ID: US 5393530 A

L2: Entry 15 of 18 File: USPT Feb 28, 1995

US-PAT-NO: 5393530
DOCUMENT-IDENTIFIER: US 5393530 A

TITLE: Method for making liposomes of enhanced entrapping capacity toward foreign substances to be encapsulated

DATE-ISSUED: February 28, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schneider; Michel	Troinex			CHX
Tournier; Herve	Valleiry			FRX
Hyacinthe; Roland	Aubonne			FRX
Guillot; Christian	Le Chable-Beaumont			FRX
Lamy; Bernard	Geneva			CHX

US-CL-CURRENT: 424/450; 264/4.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 16. Document ID: US 5389378 A

L2: Entry 16 of 18 File: USPT Feb 14, 1995

US-PAT-NO: 5389378
DOCUMENT-IDENTIFIER: US 5389378 A

TITLE: Benzoporphyrin vesicles and their use in photodynamic therapy

DATE-ISSUED: February 14, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Madden; Thomas D.	Vancouver			CAX

US-CL-CURRENT: 424/450; 428/402.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 17. Document ID: US 4911929 A

L2: Entry 17 of 18 File: USPT Mar 27, 1990

US-PAT-NO: 4911929
DOCUMENT-IDENTIFIER: US 4911929 A

TITLE: Blood substitute comprising liposome-encapsulated hemoglobin

DATE-ISSUED: March 27, 1990

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Farmer; Martha C.	Washington	DC		
Beissinger; Richard L.	Oak Park	IL		

US-CL-CURRENT: 424/450; 428/402.2, 436/829, 514/6, 514/832, 514/833

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 18. Document ID: US 4776991 A

L2: Entry 18 of 18 File: USPT Oct 11, 1988

US-PAT-NO: 4776991
DOCUMENT-IDENTIFIER: US 4776991 A

TITLE: Scaled-up production of liposome-encapsulated hemoglobin

DATE-ISSUED: October 11, 1988

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Farmer; Martha C.	Washington	DC		
Beissinger; Richard L.	Oak Park	IL		

US-CL-CURRENT: 264/4.3; 264/4.1, 264/4.6, 424/450, 428/402.2, 436/829, 514/6, 514/832, 514/833

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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L2: Entry 15 of 18 File: USPT Feb 28, 1995

DOCUMENT-IDENTIFIER: US 5393530 A
TITLE: Method for making liposomes of enhanced entrapping capacity toward foreign substances to be encapsulated

Brief Summary Paragraph Right (7):
According to another route for filling liposomes with foreign non-lipidic substances, conditions are provided under which such substances can penetrate into the vesicle core through its walls; this technique, called "transmembrane loading", involves internalizing the substances to be encapsulated into the liposome vesicles after the latter have been formed. Normally, the crossing over of the lipid membrane by foreign substances (particularly ionic) is difficult because the incoming substances are repelled by the polar groups of said lipids. However this effect can be minimized by incorporating "shield" carriers to the lipid membrane. For instance, liposomes can be loaded with cations at room temperature when the lipid membrane contains a lipophilic carrier such as acetylacetone (BEAUMIER et al., J. Nucl. Med. 32 (1982) 810). Otherwise, foreign substances may be internalized into liposomes by osmotically controlled permeation through the lipidic membrane wall. For instance, the uptake of foreign substances by the liposomes can be promoted by a transmembrane ionic gradient, e.g. a Na.sup.+ /K.sup.+ gradient as disclosed in J. Biol. Chem. 260 (1985), 802-808. A pH gradient is also effective for promoting transmembrane loading as mentioned in Biochim. Biophys. Acta 857 (1986), 123-126, WO-A-89/04656 and PCT/US85/01501. However, this technique is limited to some specific categories of drugs, more particularly weak bases, as acknowledged in Chem. Phys. Lipids 53 (1990), 37. Furthermore, making liposomes in a carrier phase of pH different from that of the core phase is difficult and, in addition, too low or too high a pH may cause membrane damage due to premature hydrolysis of the lipids.

Brief Summary Paragraph Right (8):
In EP-A-361.894 (THE HEBREW UNIVERSITY), there is disclosed a technique in which amphipatic drugs are loaded into liposomic vesicles by transmembrane internalization under the control of a post-generated pH gradient. The key feature of this technique depends on the leakage of ammonia (NH.sub.3) from the core of liposome vesicles loaded with an aqueous solution of an ammonium compound and placed in an ammonium-free carrier medium. Leakage of NH.sub.3 from NH.sub.4.sup.+ releases a proton with consecutive lowering of the pH of the entrapped liquid and consecutive establishment of a pH gradient across the liposome membrane, i.e. the carrier liquid becomes alkaline relative to the internal content of the liposome core. When an amphipatic compound (e.g. a drug with a deprotonated amine group) is added to the "alkalinized" carrier liquid, the system will tend to reequilibrate and a diffusion of said amphipatic compound into the core of the liposomes through the lipid membrane will occur.

Brief Summary Paragraph Right (9):
Techniques in which dehydrated and rehydrated liposomes are subjected to transmembrane loading also exist. For example, U.S. Pat. No. 4,673,567 (SHIONOGI & Co.) discloses preparing "empty" MLV liposomes in an ion-free aqueous carrier liquid and dehydrating these liposomes by lyophilization; then the dried liposomes are rehydrated by suspending in a carrier liquid containing a drug like Fluorouracil, Cefalexin or the like, and incubation is carried out by heating for 5 min at 50.degree. C., whereby a significant portion of the drug dissolved in the carrier liquid becomes entrapped in the liposomes. The rationale behind this approach is that "freeze-drying liposomes produces structural defects in the bilayer membrane and

heating above the transition temperature removes these defects" as acknowledged in an article by H. JIZOMOTO et al. in Chem. Pharm. Bull. 37 (1989), 3066-3069. However, as indicated in U.S. Pat. No. 4,673,567, this method is hampered by a considerable reduction in the captured volume when the carrier liquid contains ionic solutes. For instance, from the data reported in Table 1, col.3 of this document, when using isotonic brine or 0.02 phosphate buffer as the carrier liquid, the transmembrane drug take-up was practically negligible, whereas when the drug was dissolved in pure Water a value of captured volume of 16.6 .mu.l/mg of lipid was reported. Furthermore, it should be realized that in current practice, high values of captured volumes are not easily attainable. For instance, in a recent survey article: "The accumulation of drugs within large unilamellar vesicles exhibiting a proton gradient", by T. D. MADDEN & al., in Chemistry and Physics of Lipids 53 (1990), 37-46, the quoted captured value does not exceed about 1-2 .mu.l/mg of phosphatidylcholine.

Detailed Description Paragraph Right (10):
MLV liposomes were prepared in distilled water as described in Example 1. Prior to extrusion, the liposome suspension was repeatedly frozen (at -75.degree. C.) and thawed (at 40.degree. C.) four times according to the method of Mayer et al. (Biochim. Biophys. Acta 817 (1985), 193). Both extruded (2 .mu.m) and non-extruded liposome suspensions were prepared and incubated at 60.degree. C. for 30 min. with a Iopamidol solution (260 mg iodine/ml). Extruded liposomes gave an I/L value of 5, whereas non-extruded liposomes gave an I/L value of 6.3. When, in a variant, extrusion was performed below the lipids transition temperature, e.g. at from room temperature to 50.degree. C., higher entrappment yields (I/L=8 or more) were recorded.

CLAIMS:

4. The method of claim 3, wherein a temperature of the liposome suspension being forced through the membrane is below the lipid transition temperature T.sub.c.

L2: Entry 10 of 18 File: USPT Oct 13, 1998

DOCUMENT-IDENTIFIER: US 5820848 A
TITLE: Methods of preparing interdigitation-fusion liposomes and gels which encapsulate a bioactive agent

Brief Summary Paragraph Right (5):
A number of methods are presently available for "charging" liposomes with bioactive agents (see, for example, Rahman et al., U.S. Pat. No. 3,993,754; Sears, U.S. Pat. No. 4,145,410; Papahadjopoulos, et al., U.S. Pat. No. 4,235,871; Lenk et al., U.S. Pat. No. 4,522,803; and Fountain et al., U.S. Pat. No. 4,588,578). Ionizable bioactive agents have been shown to accumulate in liposomes in response to an imposed proton or ionic gradient (see, Bally et al., U.S. Pat. No. 5,077,056; Mayer, et al. (1986); Mayer, et al. (1988); and Bally, et al. (1988)). Liposomal encapsulation could potentially provide numerous beneficial effects for a wide variety of pharmaceutical agents and a high drug to lipid ratio should prove important in realizing the potential of liposomally encapsulated agents. Full references for these citations are provided on the attached form; the contents of their disclosures are incorporated herein by reference.

Detailed Description Paragraph Right (47):
Liposomes (LUVS, MLVs, SPLVs, etc. as prepared above) comprising a saturated lipid such as dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine or distearoyl phosphatidylcholine are prepared and sized to 1 micron or less (preferably, 0.025 microns) by extrusion, sonication or homogenization. A bioactive agent which does not interact with the lipid is then mixed in with the aqueous solvent used to form the Liposomes (final lipid concentration should be about 5 to 100 mM, preferably about 10 to 35 mM). The temperature of the liposomes are below the main phase transition temperature of the lipid. Thereafter, ethanol is added to a final concentration of about 1.75 to about 2.5M in the aqueous solvent. In the case of bioactive agents which interact with the lipid, the agents are generally added to the solvent after the addition of ethanol and formation of the IF gel. At this stage, the procedure can be stopped and the resulting gel used in topical and oral formulations, Alternatively, the gel may be used to form IF liposomes of the present invention.

Detailed Description Paragraph Right (123):
The results are presented graphically in FIG. 13 for the DPPC tests, and in FIG. 14 for the DSPC tests. For DPPC, as shown in FIG. 13, samples pressurized below the phase transition temperature of the lipid (41.degree. C. for DPPC) were gel like in appearance--just like the gels resulting from ethanol addition below the phase transition temperature. The viscous nature of these samples disappeared after they were heated above 41.degree. C. For DSPC, as shown in FIG. 14, samples pressurized below the phase transition temperature of the lipid (54.degree. C. for DSPC) were gel like in appearance. The viscous nature of these samples disappeared after they were heated to 70.degree. C. In both cases, heating resulted in transformation from interdigitated sheets to liposomes.

L2: Entry 7 of 18 File: USPT Aug 17, 1999

DOCUMENT-IDENTIFIER: US 5939096 A
TITLE: Liposome drug-loading method and composition

Abstract Paragraph Left (1):
A method of stably encapsulating a weak acid drug in liposomes, at a high concentration, is disclosed. The method employs a proton shuttle mechanism involving the salt of a weak acid to generate a higher inside/lower outside pH gradient. The weak acid compound accumulates in liposomes in response to this gradient, and may be retained in the liposomes by cation-promoted precipitation or low permeability across the liposome transmembrane barrier. Also disclosed is a reagent combination for practicing the method, and a liposome composition formed by the method.

Brief Summary Paragraph Right (35):
In the case of ionizable hydrophilic or amphipathic drugs, even greater drug-loading efficiency can be achieved by loading the drug into liposomes against a transmembrane pH gradient (Nichols, et al., 1976; Cramer, et al., 1977). Typically the drug contains an ionizable amine group, and is loaded by adding it to a suspension of liposomes prepared to have a lower inside/higher outside pH gradient. Although high drug loading can be achieved by this approach (e.g., U.S. Pat. No. 5,077,056), the drug tends to leak out over time as the liposome transmembrane proton gradient decays.

Brief Summary Paragraph Right (36):
The latter problem has been addressed, for drugs having an ionizable amine group, by loading the drug across an ammonium ion gradient (Haran, et al., 1993). Ammonium ions within the liposomes are in equilibrium with ammonia, which is freely permeable through the liposome membrane, and protons, which therefore accumulate as ammonia is lost from the liposomes, leading to a lower inside/higher outside pH gradient. After establishing the gradient, excess ammonium ions within the liposomes provide a reservoir of protons, to maintain the liposome pH gradient over time. This approach, however, is limited to drugs which are positively charged in their ionized state.

Brief Summary Paragraph Right (38):
The present invention includes, in one aspect, a method of forming liposomes having a higher inside/lower outside pH gradient. The gradient is established by preparing a suspension of liposomes in an aqueous solution containing a salt of a weak acid which is capable of freely permeating the liposome membrane. The suspension is then treated to produce a higher inside/lower outside concentration gradient of the weak acid. The weak acid is allowed to distribute between inner and outer compartments, acting as an inside-to-outside proton shuttle to generate a higher inside/lower outside pH gradient.

Brief Summary Paragraph Right (40):
In a related aspect, the invention provides a method for loading a weak-acid compound into liposomes having a higher inside/lower outside pH gradient. Loading is carried out by adding the weak acid compound to a suspension of liposomes having a higher inside/lower outside gradient of a salt of a weak acid which includes the given cation. The protonated form of the weak acid salt acts as an inside-to-outside proton shuttle to generate a higher inside/lower outside pH gradient to drive loading of the weak acid compound into the liposome interior.

Brief Summary Paragraph Right (43):

In another general embodiment, the protonated form of the weak acid compound is readily able to permeate the liposome transmembrane barrier only at a temperature above the phase transition temperature of the liposomes. The compound is loaded at a temperature above this phase transition temperature, and the suspension is stored at a temperature below the phase-transition temperature.

Brief Summary Paragraph Right (44):
In still another aspect, the invention includes a reagent combination for use in producing a suspension of liposomes with an encapsulated weak-acid compound. The combination includes liposomes of the type described above, having a higher inside/lower outside gradient of a weak acid, the weak acid compound to be loaded, and means for retaining compound in the liposomes after compound loading.

Drawing Description Paragraph Right (4):
FIG. 3 is a schematic illustration of the loading of a weak acid drug, "D--COOH", into liposomes against a higher inside/lower outside pH gradient established by the method of the present invention;

Drawing Description Paragraph Right (7):
FIG. 6A is a gel exclusion chromatography profile of higher inside/lower outside acetate gradient liposomes incubated with 5(6)-carboxyfluorescein for either 17 hours (broken line) or 0 hours (solid line-control) at 70.degree. C.;

Detailed Description Paragraph Right (10):
A "higher inside/lower outside pH gradient" refers to a transmembrane pH gradient between the interior of liposomes (higher pH) and the external medium (lower pH) in which the liposomes are suspended. Typically, the interior liposome pH is at least 1 pH unit greater than the external medium pH, and preferably 2-4 units greater.

Detailed Description Paragraph Right (11):
This section describes the preparation of a suspension of liposomes having a higher inside/lower outside pH gradient, in accordance with the invention.

Detailed Description Paragraph Right (30):
C. Formation of Liposome pH Gradient

Detailed Description Paragraph Right (31):
After sizing, the external medium of the liposomes is treated to produce a higher inside/lower outside concentration gradient of the weak acid salt. This may be done in a variety of ways, e.g., by (i) diluting the external medium, (ii) dialysis against the desired final medium, (iii) molecular-sieve chromatography, e.g., using Sephadex G-50, against the desired medium, or (iv) high-speed centrifugation and resuspension of pelleted liposomes in the desired final medium.

Detailed Description Paragraph Right (34):
After adjusting the external medium to produce a higher inside/lower outside concentration gradient of the weak acid salt, the weak acid is allowed to distribute between inner and outer liposome compartments, with the weak acid salt acting as an inside-to-outside proton shuttle, until an equilibrium higher inside/lower outside pH gradient is formed.

Detailed Description Paragraph Right (35):
FIG. 1 illustrates the proton-shuttle mechanism by which the pH gradient is formed. The figure shows a liposome 10 having a bilayer membrane 12 and having encapsulated therein, the salt of a weak acid, in this case, the calcium salt of acetate, with the acetate anion being in equilibrium with the uncharged (protonated) form of the acid. The bilayer membrane serves as a partition between the liposome inner compartment, indicated at 14, and an outer bulk phase suspension medium 16.

Detailed Description Paragraph Right (36):
As indicated, acetate is effectively impermeant to the liposome membrane in its charged form, but readily passes through the membrane in its protonated form. Because of the weak-acid gradient across the liposome membrane, protonated weak acid shows a net migration in an inside-to-outside direction, acting to shuttle protons out of the liposomes. This leads to an increased concentration of hydroxyl ions inside the

liposomes and an increased proton concentration outside the liposomes.

Detailed Description Paragraph Right (46):
It will be appreciated from the above that the pH gradient across the liposomes is self-regulating and self-sustaining, i.e., not degraded by leakage of protons into the liposomes or hydroxyl ions out of the liposomes after the gradient is formed. This feature can be appreciated from the proton shuttle mechanism illustrated in FIG. 1. Here it is assumed that a pH gradient has been established and the liposomes are stored over an extended period in suspension. During storage, as hydroxyl ions leak out from the liposomes into the external medium, and as protons leak into the liposomes from the external medium, the equilibrium between charged and protonated form of the weak acid (acetate) in the liposomes shifts toward the protonated form, increasing the level of proton shuttling out of the liposomes, acting to restore the pH gradient.

Detailed Description Paragraph Right (47):
The pH gradient liposomes formed as above are used in loading a weak-acid compound into the liposomes, according to another aspect of the invention. In this method, the compound is added to a suspension of the pH gradient liposomes, and the suspension is treated under conditions effective to load weak acid compound within the liposomes.

Detailed Description Paragraph Right (49):
FIG. 3 illustrates the mechanism of drug loading into liposomes, in accordance with the method. The figure shows a liposome 18 having a bilayer membrane 20 and a higher inside/lower outside pH gradient, by virtue of a higher inside/lower outside gradient of a weak acid, i.e., the anion of the weak acid, in this case the acetate anion.

Detailed Description Paragraph Right (50):
The bottom of the figure shows the mechanism by which a higher inside/lower outside pH gradient is formed, as described in Section II. The upper part of the figure shows the mechanism of loading of the weak acid compound, indicated as "D--COOH". The compound, which is present originally only in the external compartment, is shown in equilibrium in this compartment between negatively charged and uncharged, protonated forms, with lower pH favoring the latter form. As indicated, the compound is able to pass through the liposome membrane only in its protonated form. In the absence of a pH gradient, the compound would equilibrate to equal concentrations on both sides of the membrane. Because of the higher internal pH, the equilibrium between the charged and uncharged form of the compound is shifted toward the charged, nonpermeable form, leading to net loading of the compound in the liposomes. Assuming the compound remains in solution in its liposome-loaded form, the extent of liposome loading for weak acid compounds is governed by the Henderson-Hasselbach relationship:

Detailed Description Paragraph Right (51):
By way of example, with a pH gradient of 4 pH units, and an outside-to-inside volume ratio of 100:1, a theoretical loading factor of 100:1 inside:outside is possible. Based on these considerations alone, it will be appreciated that it is possible to achieve substantially 100% loading efficiency, i.e., loading of substantially all of the compound present into the liposomes, by proper selection of the initial external concentration of compound in relation to the known inside/outside volume ratio of the liposomes, which can be estimated.

Detailed Description Paragraph Right (55):
In some cases, the weak acid compound, in associated form, is readily able to permeate the liposome transmembrane only at a temperature above the phase transition temperature of the liposomes. In such cases, loading of the compound into the liposomes is carried out at a temperature above the liposome phase transition temperature, and entrapment of the compound is effected by cooling the liposome suspension containing encapsulated compound below the lipid phase transition temperature. Entrapment carried out as described above offers prolonged stability to the encapsulated compound, particularly against degradative processes which might otherwise impact the compound in non-encapsulated form.

Detailed Description Paragraph Right (57):
Liposomes having a higher inside/lower outside pH gradient in response to a transmembrane difference in acetate ion concentration are prepared as described

above. Typically, weak acid compounds to be loaded are added to the bulk medium at concentrations ranging from 1 .mu.M-100 mM, with the concentration selected depending upon both the absolute quantity of drug intended for encapsulation and the degree of loading efficiency desired, as discussed above.

Detailed Description Paragraph Right (58):
After adding the weak acid compound to the liposomes, the liposomes are treated under conditions effective to trap the compound within the liposomes. Conditions suitable for compound loading are those which (i) allow diffusion of the weak acid compound, with such in an uncharged form, into the liposomes, (ii) lead to a desired final loading concentration and efficiency, and (iii) provide a self-sustaining pH gradient after drug loading.

Detailed Description Paragraph Right (59):
Considering the first of these requirements, the loading period may range from 1 minute to several hours, and is typically between 15-120 minutes, depending on permeability of the weak acid drug into the liposomes, temperature, and the relative concentrations of liposome lipid and drug. Where the compound is one which readily permeates the liposome membrane only above the phase transition temperature of the liposome lipids, e.g., 50.degree. C., the loading is carried out above this temperature. After loading, the liposomes are cooled below the phase transition temperature, e.g., to a storage temperature between 4-24.degree. C., such cooling acting to retard efflux of the loaded compound from the liposomes, independent of a pH gradient mechanism.

Detailed Description Paragraph Right (60):
The final drug loading concentration and loading efficiency may be approximated from the Henderson-Hasselbach relationship, as discussed above. In addition to the considerations already discussed, the concentration of weak acid remaining after drug loading must be sufficient to maintain a high inside/low outside concentration gradient of the weak acid, preferably a ratio of at least 10:1. Thus, for example, if the initial concentration of weak acid is 150 mM, and the final concentration of loaded weak-acid compound is 50 mM, the final concentration of weak acid in the liposomes would be 100 mM (ignoring the loss of weak acid used in establishing the pH gradient), since capture of each molecule of compound within the liposomes, by deprotonating the compound, requires the shuttling of one proton out of the liposomes, and thus the efflux of one molecule of the weak acid from the liposomes. Assuming a V.sub.o /V.sub.i ratio of 50, concentration gradient of weak acid after drug loading in this example would be 100:50/50, or 100:1, sufficient to maintain a inside to outside drug loading ratio of 100:1.

Detailed Description Paragraph Right (61):
In addition, the excess weak acid in the liposomes after drug loading provides a reservoir for sustaining the pH gradient across the liposomes over an extended storage time, as the equilibrium between protonated and unprotonated forms of the encapsulated weak acid is shifted in response to hydroxyl ion efflux or proton influx over time, as described in Section II. Accordingly, drug efflux from the liposomes on storage is effectively uncoupled from proton influx or hydroxyl-ion efflux, allowing for stable compound storage in suspension form over an extended period.

Detailed Description Paragraph Right (63):
To illustrate one embodiment of the present invention, two exemplary weak acids were loaded into pH gradient liposomes. The compounds were loaded using as a driving force the pH gradient generated by a transmembrane difference in acetate concentrations, as described in Example 3. The model compounds selected for loading, 5(6)-carboxyfluorescein and nalidixic acid, are both fluorescent weak acid compounds. Their fluorescent properties provided a useful means for determining the concentration of the compounds in liposomal media.

Detailed Description Paragraph Right (66):
The loading behavior of the weak acid compounds in response to both acetate gradient and non-gradient liposomes is illustrated in FIGS. 5A, 5B and is described in Example 3B.

Detailed Description Paragraph Right (67):

FIG. 5A illustrates the loading behavior of 5(6)-carboxyfluorescein. Briefly, incubation of a solution of 5(6)-carboxyfluorescein in aqueous calcium acetate (solid line/boxes) with acetate-loaded liposomes resulted in no change in external concentration of the weak acid drug, suggesting that in the absence of a transmembrane ion gradient, the driving force for remote loading of the weak acid compound is removed. In contrast, when the acetate loaded liposomes are incubated in an aqueous sodium sulfate solution containing 5(6)-carboxyfluorescein (dotted line/circles), a rapid decrease in fluorescence was observed, indicating the uptake of the fluorescent weak acid compound into the liposome interior.

Detailed Description Paragraph Right (68):
Similar results were observed for nalidixic acid (FIG. 5B) and indicate that in the absence of an acetate ion gradient, weak acid compounds are not effectively loaded into the liposome interior. The amount of weak acid compound that can be loaded increases with increasing transmembrane ion differential.

Detailed Description Paragraph Right (70):
The weak acid compounds, 5(6)-carboxyfluorescein and nalidixic acid, were each loaded into acetate gradient liposomes as detailed in Example 3C. 5(6)-carboxyfluorescein was incubated with acetate gradient liposomes for 17 hours (FIG. 6A, circles) or for 0 hours (control, squares) at 70.degree. C. Nalidixic acid was similarly incubated with acetate gradient liposomes for 15 minutes at 25.degree. C. (FIG. 6B, squares). The amount of each of the weak acid compounds entrapped in the liposome interior was directly determined by first separating trapped material from free material by gel exclusion chromatography, followed by detergent-promoted release of entrapped compound with Triton X100. The fluorescence intensity of each fraction was measured following detergent treatment.

Detailed Description Paragraph Right (72):
A similar analysis of nalidixic acid revealed that seventy two percent of the total fluorescence was liposome-associated (FIG. 6B, squares). To further quantitate the amount of weak acid compound loaded into acetate gradient liposomes, the molar ratio of nalidixic acid to phospholipid was determined as described in Example 3C. As shown in FIG. 6B, the molar ratio of nalidixic acid to phospholipid (dotted line/circles) was determined to be about 4.times.10.sup.-3. This corresponds to an internal concentration of nalidixic acid of about 1 mM, about ten times larger than the initial external concentration of weak acid compound.

Detailed Description Paragraph Right (74):
However, with proper selection of liposome concentration, external concentration of added compound, and the pH gradient, essentially all of the weak acid compound may be loaded into the liposomes. For example, with a pH gradient of 2-3 units (or the potential of such a gradient employing an acetate ion gradient), the final internal:external concentration of drug will be about 1000:1. Knowing the calculated internal liposome volume, and the maximum concentration of loaded drug, one can then select an amount of drug in the external medium which leads to substantially complete loading into the liposomes.

Detailed Description Paragraph Right (78):
Another approach for releasing entrapped compound is to equilibrate the concentrations of acetate counterion (in this case, calcium) across the transmembrane barrier. As described in Example 3F, calcium acetate loaded liposomes were added at t=100 seconds to solutions of nalidixic acid maintained at 25.degree. C. (FIG. 8, solid line) and 60.degree. C. (broken line), respectively. The decrease in fluorescence was monitored over time, indicated loading of compound into the acetate gradient liposomes.

Detailed Description Paragraph Right (79):
The experiment was carried out at two different temperatures to investigate how changes in lipid phase (i.e., gel versus liquid-crystalline) affects the penetration of molecules into the bilayer. When the suspension was incubated at 25.degree. C. (i.e., a temperature below the lipid phase transition temperature), addition of the ionophore at t=700 seconds had no effect on the fluorescence, as shown in FIG. 8 (solid line).

Detailed Description Paragraph Right (95):
In the most general embodiment, the retaining means includes the weak-acid transmembrane gradient which is due to an excess of weak acid species in the liposomes after drug loading, and which provides a reservoir for sustaining the pH gradient, as discussed above.

Detailed Description Paragraph Right (96):
Where the loaded compound is one which does not readily permeate the liposome membranes below the lipid phase transition temperature of the liposomes, the retaining means may additionally include the low-permeability barrier provided by the lipid bilayer.

Detailed Description Paragraph Right (97):
Finally, where the loaded compound is one which has a low solubility in the presence of a selected cation, the cation itself provides retaining means by holding loading compound in a precipitated form that prevents efflux from the liposomes. In particular, it will be appreciated that the precipitating mechanism allows higher amounts of compound to be loaded stably into liposomes than is possible by a pH gradient alone, since the Henderson-Hasselbach relationship applies only to the solute form of the compound. For example, if the loaded compound precipitated above 5 mM compound concentration in the liposomes, a gradient effective to load to just above this relatively low concentration would be effective to load the liposomes to a high total compound concentration, e.g., 100-200 mM.

Detailed Description Paragraph Right (99):
Also included in the invention is the compound-loaded liposome composition formed by the above method, for use in delivering the loaded weak-acid compound. The composition includes, in an aqueous-suspension form, weak-acid gradient liposomes of the types described in Section II, the weak-acid compound encapsulated in the liposomes, by the loading method described in Section III, and retaining means in the liposomes for retaining the compound encapsulated in the liposomes, also as described in Section III.

Detailed Description Paragraph Right (101):
It will be appreciated how the features of the invention contribute to its applications in drug-delivery or other uses of compound-loaded liposomes. The weak-acid gradient liposomes used for compound loading are effective to generate their own pH gradient, and self-sustain this gradient by the shifting equilibrium between protonated and non-protonated forms of the encapsulated weak acid.

Detailed Description Paragraph Right (102):
Loading to high drug concentrations, and high efficiencies can be achieved by proper selection of external concentration of compound in relation to the weak-acid gradient and internal:external volume ratio in the liposome suspension. This loading can be carried out at a manufacturing site, or remotely at the site of use.

Detailed Description Paragraph Right (103):
The liposomes, once loaded, are capable of retaining the compound at high concentration over an extended storage and/or drug-delivery period, by virtue of the self-sustaining gradient mechanism provided by the reservoir of weak acid in the liposomes. When coupled with other retaining means, including the use of high phase transition lipids and/or low compound solubility in the presence of the weak acid counterion, stable compound loading for periods of up to several months in suspension form may be achieved.

Detailed Description Paragraph Right (120):
Two weak acid compounds, 5(6)-carboxyfluorescein and nalidixic acid, were selected for remote loading into pH gradient liposomes. Properties of the weak acids are given in Table II below.

Detailed Description Paragraph Center (3):
II. Preparation of pH Gradient Liposomes

Detailed Description Paragraph Center (9):
Formation of Liposomes Having a Transmembrane Acetate Gradient

Detailed Description Paragraph Center (11):
Gradient Loading of (6)-Carboxyfluorescein and Nalidixic Acid into Liposomes

Other Reference Publication (10):
Deamer, D., et al. "The Response of Fluorescent Amines to ph-Gradients Across Liposome Membranes," Biochem et Biophysica Acta 274: 323-335 (1972).

Other Reference Publication (16):
Nichols, J., et al., "Catecholamine Update and Concentration By Liposomes Maintaining pH Gradients," Biochim. Biophys. Acta 455: 269-271 (1976).

CLAIMS:

1. A method of forming liposomes having a higher inside/lower outside pH gradient, comprising:

preparing a suspension of liposomes in an aqueous solution of a weak acid salt comprising (i) an anion, which, in protonated form, is uncharged and is capable of freely permeating the transmembrane barrier of liposomes, and (ii) a counterion that is substantially lipid membrane impermeable,

adjusting the concentration of weak acid salt present in the external medium to produce a higher inside/lower outside concentration gradient of the weak acid salt, and

allowing the weak acid to distribute itself between inner and outer liposome compartments, with the weak acid acting as an inside-to-outside proton shuttle, thereby generating a higher inside/lower outside pH gradient.

6. A method of loading a weak-acid compound into liposomes, comprising:

adding the compound to a suspension of liposomes having a higher inside/lower outside gradient of a weak acid salt comprising (a) an anion, which, in protonated form is uncharged and is capable of readily permeating the transmembrane barrier of the liposomes, and (b) a counterion that is substantially lipid membrane impermeable, wherein the weak acid acts as an inside-to-outside proton shuttle to generate a higher inside/lower outside pH gradient and an accumulation of the compound within the liposomes, and

by said adding, achieving uptake of the compound within the liposomes.

11. The method of claim 6, wherein (i) the compound in protonated form is readily able to permeate the liposome transmembrane barrier only at a temperature above the phase transition temperature of the liposomes, (ii) the compound and suspension are maintained at a temperature above the phase transition temperature during compound accumulation into the liposomes, and (iii) said method further comprises cooling the compound and suspension below such transition temperature after compound loading into the liposomes.

WEST Search History

DATE: Tuesday, July 09, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=OR			
L2	L1 and (below adj3 transition adj1 temperature)	18	L2
L1	liposome$ same gradient$	580	L1

END OF SEARCH HISTORY